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Transport and Retention of Manure-Borne Coliforms in Soil

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ABSTRACT

Manure is a source of several bacterial pathogens that can potentially contribute to surface and groundwater contamination. Results from most bacterial transport studies in soils are only partially applicable to manure-borne bacteria because microorganisms are released along with manure particulates as manure dissolves. The objective of this study was to compare transport of chloride ion, *Escherichia coli* (*E. coli*) and manure colloids in undisturbed soil columns with a well-developed structure. Breakthrough column experiments were conducted with undisturbed, 20-cm long Tyler soil columns from the A horizon. A pulse of 4% filtered bovine manure solution with *E. coli* and KCl was passed through the columns. *Escherichia coli* concentrations, chloride content, and turbidity were measured in influent and in effluent. Columns were cut into 2-cm layers after the experiment to measure: (i) viable bacterial concentrations in pore solution and attached to soil; (ii) bulk density; (iii) water content. Companion batch experiments were performed to measure attachment of *E. coli* to soil in the presence of various amounts of manure. *Escherichia coli* attachment to soil decreased with increased manure content due to increased competition for attachment sites. Flow velocity affected *E. coli* transport and attachment to soil; there was relatively more attachment at slower flow velocity than at higher flow velocity. *Escherichia coli* attachment to soil was 18, 5, and 9% at flow velocities of 2.3, 8.4, and 9.3 cm d^{-1}, respectively. Spatial variability in soil structure may result in large variations of pore water velocity and consequent differences in transport of manure particulates and bacteria under ponded infiltration.

Soil can serve as an effective bacterial filter. Bacteria retardation properties of soils and sediments have been reported in numerous studies. Aulenbach et al. (1974) reported that 99% of the coliforms from secondary effluent were removed by movement through 3 m of sand. Bouwer et al. (1974) observed about a 4 log reduction in fecal coliform concentration in water from a 9.1 m deep well below loamy sand recharge basins flooded with sewage effluent. Jones (1968) concluded that the coliform bacteria travel distance did not exceed 31 m in fine sand. Dazzo et al. (1973) found that only 10% of the fecal coliforms present in fresh dairy manure percolated below 13-cm depth in soil; no coliforms were detected below a depth of 48 cm. Lance et al. (1976) found that filtration through 250-cm long columns filled with loamy sand reduced fecal coliform concentrations by about 3 logs during a 9-d flooding period. Reneau et al. (1975) concluded that soil layers restricted coliform bacteria movement to the groundwater system, and that the groundwater quality from the watershed would improve with distance from the pollution source as a result of dilution, sedimentation, and bacteria die-off.

Although soil can mitigate bacterial movement or leaching, some bacteria applied onto soil or released within soil may still be transported through and travel in the vadose zone to groundwater. Much literature documents bacterial transport from a few meters to 830 m depending on soil or sediment texture and permeability, water saturation degree, and length of time. Stoddard et al. (1998) showed that fecal bacteria were transported to a depth of 90 cm in silt loam soil by the first rain after application of dairy manure, when <2 cm of rain had fallen during a single event. Rainfall or irrigation at intensity of 1 cm h^{-1} appeared to be sufficient to transport bacteria in poultry manure on the surface of soil block to a depth of at least 32.5 cm in the silt loam soil as result of preferential flow in well-structured soil (McMurry et al., 1998). In tilled blocks, the fecal coliform concentrations took longer to elute because preferential flow paths were disrupted in the upper 12.5 cm of the soil. Rainfall on well-structured soil caused the preferential movement of fecal bacteria even in unsaturated flow conditions (McMurry et al., 1998). Hagedorn et al. (1978) observed *E. coli* and *Streptococcus fecalis* transport in eight directions from pits filled with topsoil and gravel after two heavy rains. The strong directional subsurface bacteria movement was more rapid than had been expected for 2% surface gradient. Smith et al. (1985) studied *E. coli* in columns filled with either undisturbed or sieved through a 20-mm screen and repacked silt loam soil at different flow rates. Disturbed cores retained at least 93% of the cells applied compared with 21 to 78% in intact cores. The authors suggested that macropore transport had contributed to the rapid bacterial movement. Natsch et al. (1996) studied bromide and a biocontrol strain of *Pseudomonas fluorescens* transport in structural clayey loam soil (gleyic Cambisol). They concluded that if rainfall occurred immediately after release of bacteria into field soil, macropore flow could lead to the displacement of a significant number of the applied bacteria to at least 150-cm deep. Unc and Goss (2003) studied vertical transport of coliforms from liquid swine and solid cow manure applied on the surface of silty loam and sandy loam soil in southern Ontario. They found that the velocity of bacteria transport was 35 times greater than the average pore-water velocity in soil and they attributed such transport to presence of macropores.

Manure application and animal grazing lead to application of large amounts of microorganisms on soil surfaces. Microorganisms, including human pathogens, are

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Abbreviations: BTCs, breakthrough curves; CBTC, cumulative breakthrough curve; CFUs, colony forming units; EC, electrical conductivity; NTU, nephelometric turbidity units.

Table 1. Texture, aggregate size distribution, and soil organic carbon in soil columns.

Column	Particle size			Aggregates						
	Clay	Silt	Organic C†	2–4 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	0.125–0.25 mm	0.063–0.125 mm	<0.063 mm
	%									
						0–10 cm				
1	24.2	46.2	4.29	45.8	27.9	18.7	2.8	1.7	1.1	2.0
2	26.2	46.0	3.82	40.5	30.3	20.1	3.4	2.1	1.4	2.2
3	27.9	47.6	3.26	52.4	25.8	15.3	2.2	1.4	0.9	2.0
						10–20 cm				
1	24.7	43.7	2.78	50.0	27.5	16.2	2.3	1.4	0.9	1.7
2	29.8	45.4	2.75	47.4	26.6	17.5	3.0	1.9	1.4	2.3
3	25.4	45.8	2.72	43.1	26.9	19.8	3.4	2.3	1.5	3.0

† Measured after the column experiment.

released as manure dissolves, and have a potential to reach water sources (Reddy et al., 1981; Harvey et al., 1989; Jamieson et al., 2002, Unc and Goss, 2003). As the microorganisms are released with manure particulates, a coupled transport of microorganisms and organic colloids occurs in soils. Studies of coupled microorganisms and manure transport in soils are scarce. Shelton et al. (2003) observed similarity in release and transport of fecal coliforms and manure particulates in stony soil.

The objective of this study was to document and compare transport of chloride ion, *E. coli* and manure colloids in undisturbed soil columns with well-developed structure.

MATERIALS AND METHODS

Undisturbed soil cores were taken from a well-structured A horizon of Tyler soil (fine-silty, mixed, mesic Aeric Fragiaquults) in Franklin County, Pennsylvania, at a flood-plain grazing land within a plot 0.3 m by 1 m. Cores were from the 0- to 20-cm layer and had diameter of 7.5 cm. Selected soil properties are given in Table 1. The soil was loam in four samples, and was clay loam in two samples. Clay content ranged from 24.2% in 0 to 10 cm to 29.8% in 10 to 20 cm layer.

A wild-type *E. coli* strain was isolated from bovine feces (BARC dairy herd). The culture was routinely grown overnight at 37°C in minimal lactose broth medium with yeast extract (MLB-Y). At stationary phase the cell concentration was approximately 5×10^8 cells mL^{-1} as determined using a hemocytometer chamber (Hausser Scientific, Gaithersburg, MD). Experiments were conducted with freshly grown cultures.

Viable cell counts (colony forming unit, CFU) were determined by plating on MacConkey agar using an Autoplate 4000 (Spiral Biotech, Bethsda, MA). After overnight incubation at 44°C for 16 h, colonies were counted using a QCount (Spiral Biotech).

Bacteria Attachment to Soil

Escherichia coli attachment to the soil was studied in batch experiments with disturbed soil. The procedure of the batch experiments was similar to that of Gantzer et al. (2001). Manure was collected from the Dairy Research Unit of the USDA/ARS facility in Beltsville and stored at temperature 4°C for 2 mo prior to the experiment. *Escherichia coli* was not detected in manure after 2 mo. Manure had pH of 6.95 and contained of 15.8% total solids, 1210 mg L^{-1} of total nitrogen, 306 mg L^{-1} of ammonium nitrogen (NH_4^+N), <1 mg L^{-1} of nitrate (NO_3^-), 148 mg L^{-1} of soluble phosphorus (P), and 303 mg L^{-1} of total P. The manure was filtered through cheesecloth to separate suspension from plant residue and macroscopic particles. The filtered manure was added to water to obtain water-manure suspensions of 20 000 and 40 000 mg L^{-1} liquid manure. *Escherichia coli* cells were added to the suspensions and to deionized water. The air-dry soil was passed through the 2-mm sieve. Water-manure suspensions were added to the air-dry soil with soil/suspension ratios of 2:1, 1:1, 1:2, 1:5, and 1:10 g cm^{-3}. The volume of the soil-manure suspension ranged from 12 to 52 cm^3. The soil suspensions were stirred for 30 min, and centrifuged at $1400 \times g$ for 3 min in 50 mL centrifuge tubes (PTD PRO, Elkay[1]). The above mentioned duration of stirring and centrifuging were found to be sufficient to achieve an attachment equilibrium and to allow the soil to settle in preliminary experiments (data not shown). *Escherichia coli* concentrations were measured in the supernatant and applied solutions in triplicate. The attached *E. coli* cells were calculated from the difference between the amount applied and the amount recovered in the supernatant. Temperature during the experiment was 23°C. The Freundlich isotherm equation:

$$S = K_f C^n \quad [1]$$

was fitted to data, where S is the amount of *E. coli* attached to solid phase, CFU g^{-1}; C is the concentration of *E. coli* associated with the liquid phase, CFU mL^{-1}; K_f is the Freundlich sorption coefficient equal to the attached amount at C = 1 CFU mL^{-1}; and n is the Freundlich nonlinearity parameter of the attachment.

Column Transport

Three undisturbed soil cores of 7.5 cm diam. and 20-cm length were placed into perforated PVC tubes of 25-cm length. Tubes with soil cores were submerged in paraffin through the perforation to prevent the wall filtration. Soil columns were subject to capillary saturation during 48 h. Three to five pore volumes of deionized water was passed through columns. Effluent was analyzed for the background turbidity and to confirm the absence of Cl^- and *E. coli* in pore solution before the experiment. Breakthrough experiments were performed at 9 ± 1°C to minimize bacteria growth and die-off. Bacterial suspension of concentration 4.6 10^5 CFU mL^{-1} *E. coli* and 40 000 mg L^{-1} of diluted bovine manure was prepared as described previously in the batch studies, and 164 mg L^{-1} KCl was added to the suspension to use Cl^- as a conservative tracer. The electrical conductivity (EC) of the manure suspension was measured with Solomat MPM 1000 conductivity meter (Solomat Ltd., UK). The EC value of 23.1 dS m^{-1} approximately corresponded to the value of ionic strength of 0.29 mol L^{-1} (Griffin and Jurinak, 1973). Manure suspension was

[1]Manufacturer names are given for information only, and do not constitute endorsement by the USDA.

applied to the top of columns, and a layer of suspension from 4.5 to 5 cm was kept above the soil surface. Manure suspension was removed quickly from the top of columns, and deionized water was used as the influent after 500 mL of the manure suspension infiltrated into the columns. The effluent was collected from the bottom of the columns at time intervals from 15 to 30 min with a fraction collector (Retriever II, ISCO, Inc., Lincoln, NE). The effluent volume was measured in each collected sample. Chloride content was measured with a Model 94178 chloride electrode (Thermo Electron Corp., Beverly, MA) in the detection range from 1.8 to 35 500 mg L^{-1}. Turbidity was determined using a 2020 Turbidimeter (LaMotte Co., Chestertown, MD) with the Tungsten incandescent bulb as the light source. The turbidimeter was calibrated for manure content in the range from 0 to 40 000 mg L^{-1}, and values of nephelometric turbidity units (NTU) were converted into mg L^{-1}. The *E. coli* content was determined in 50 μL subsamples that had been plated on MacConkeys Agar using an Autoplate 4000 spiral platter manufactured by Spiral Biotech (Bethesda, MD), and had been incubated for 14 h at temperature 42°C. *Escherichia coli* (CFUs) were counted using a Protocol plate reader (Synoptics, Cambridge, UK).

Soil bulk density, water content, and *E. coli* distribution were measured in columns after the experiment in 2-cm layers of soil, 10 per each column. Columns were drained overnight at 9°C on gravel filter and disassembled at 23°C. The bulk density and the water content were determined gravimetrically in a half of the soil mass at each layer. The metal ring of 2-cm height and 4-cm diam. was used to sample soil for the bulk density and the volumetric water content measurements. The *E. coli* contents associated with solid and liquid phases were measured in the soil remaining after the ring sampling. A 20-g soil sample was centrifuged using perforated tubes for 5 min at 1400 × *g*. The 5 min time duration was experimentally found sufficient to extract soil solution. The mass of extracted solution and *E. coli* content were measured. The centrifuged soil was diluted with 80 mL of deionized water, and was blended during 2 min with the Waring Commercial Laboratory Blender (Model 34BL97, Torrington, CT). The soil suspension was centrifuged at 1400 × *g* during 3 min, and *E. coli* content was determined in the supernatant. Results were converted into CFU per gram of dry soil. Soil for *E. coli* content measurements was sampled in two replications. All solutions were plated in two replications.

The aggregate size distribution was determined in soil columns in two layers from 0 to 10 cm and from 10 to 20 cm. The air dry soil was sieved through Hubbard Screen Sieves Kit, set/6#3070-6 (Hubbard Scientific, Fort Collins, CO) into the following aggregate diameter groups: <0.063, 0.063 to 0.125, 0.125 to 0.25, 0.25 to 0.5, 0.5 to 1, 1 to 2, and 2 to 4 mm. Hundred-gram soil samples were placed on the top of the set of sieves and were shaken manually during 5 min. Aggregate mass at each sieve was converted to the percentage of this aggregate fraction in total mass.

RESULTS

Escherichia coli Attachment to Soil

Manure dramatically affected *E. coli* attachment to soil at concentrations in the solution >100 CFU mL^{-1} (Fig. 1). No difference between *E. coli* content associated with the solid phase for all treatments was observed when *E. coli* concentration in the solution was <100 CFU mL^{-1}. Attachment isotherms were closer to linear without manure and were strongly nonlinear in presence

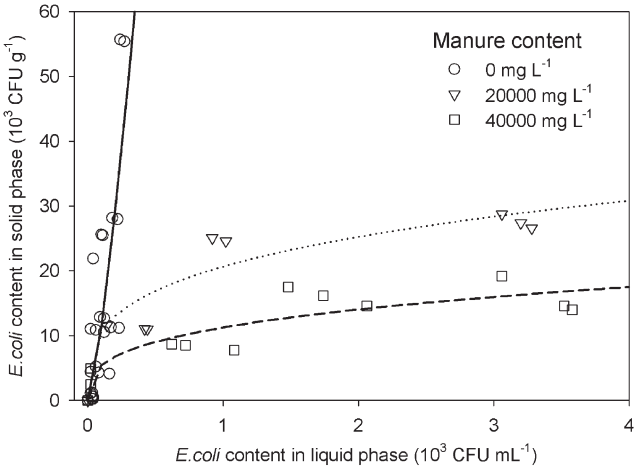

Fig. 1. Effect of dissolved manure content on *Escherichia coli* attachment to soil; symbols, observed; lines, fitted with the Freundlich isotherm equation.

of manure. The maximum *E. coli* attachment occurred in the treatment without manure. The attachment decreased with the increase of manure content (Fig. 1). The Freundlich sorption coefficient K_f was smaller and Freundlich nonlinearity parameter *n* was greater in the treatment without manure compared with treatments with manure. Values of K_f were 24.4, 2814.5, and 1249.8 in attachment experiments with 0 mg L^{-1}, 20 000 mg L^{-1}, and 40 000 mg L^{-1} manure suspensions, respectively. Values of parameter *n* were 1.335, 0.289, and 0.318, respectively.

Column Transport

The constant-height solution layer on the top of columns provided for the flow velocity about 1.25 times the value of saturated hydraulic conductivity in the columns (Table 2). Column 1 had a relatively low flow velocity of 2.26 ± 0.10 cm d^{-1}, while columns 2 and 3 had high flow velocity of 8.35 ± 0.08 and 9.28 ± 0.09 cm d^{-1}, respectively. The chloride, *E. coli,* and manure breakthrough data were expressed in terms of the relative concentration, that is, the concentration in the effluent *C* divided by the concentration in the influent C_0 vs. the number of pore volumes of the solution passed through each column. The manure concentration was not corrected for the background turbidity because the latter was negligible. The breakthrough curves (BTCs) are shown in Fig. 2. All BTC exhibited sawtooth-like oscillations. The shape of BTC for Cl^- was close to symmetrical in the column with low flow velocity (column 1), and asymmetrical in columns with high flow velocity (columns 2 and 3). The *E. coli* and manure relative concentrations in the effluent were less than those of Cl^- in all columns. Breakthrough peaks were observed first for the manure, then for the *E. coli*, and finally for Cl^- in all three columns. The maximum *E. coli* and manure effluent concentrations were observed earlier than one pore volume displacement by the influent. Concentrations of Cl^- reached maximum after one pore volume had been displaced.

Because of the oscillations, the differences in break-

Table 2. Column experiment variables.

Column	Porosity	Saturated hydraulic conductivity	Flow velocity	*E. coli* associated with			Outflow
				Soil solution	Solid phase	Total	
	$cm^3\ cm^{-3}$	$cm\ d^{-1}$		%			
1	0.420	1.81	2.26	1.2	18.1	19.3	5.8
2	0.433	6.68	8.35	1.2	5.2	6.4	32.7
3	0.445	7.42	9.28	2.8	9.2	12.0	50.0

through of chloride, *E. coli* and manure could be inspected better using cumulative BTCs, or CBTC (Fig. 3). The relative cumulative mass was computed as the cumulative mass in the effluent, M, divided by the total mass in the influent (M_0). The Cl^- and *E. coli* CBTCs flattened as the cumulative volume of effluent grew, whereas the manure CBTCs grew with an approximately constant rate. The relative mass of *E. coli* and manure in the effluent was less than the chloride mass after the half of pore volume has been displaced in columns. The manure: *E. coli*/chloride ratio of the relative mass in effluent was 0.06:0.17:1 in column 1, 0.15:0.34:1 in column 2, and 0.30:0.45:1 in column 3, respectively, at the end of the experiment.

Differences in flow velocity among columns affected transport of Cl^-, *E. coli,* and manure particulates. Figure 4 illustrates effect of the water flow velocity on the relative mass of Cl^-, *E. coli* and manure that passed

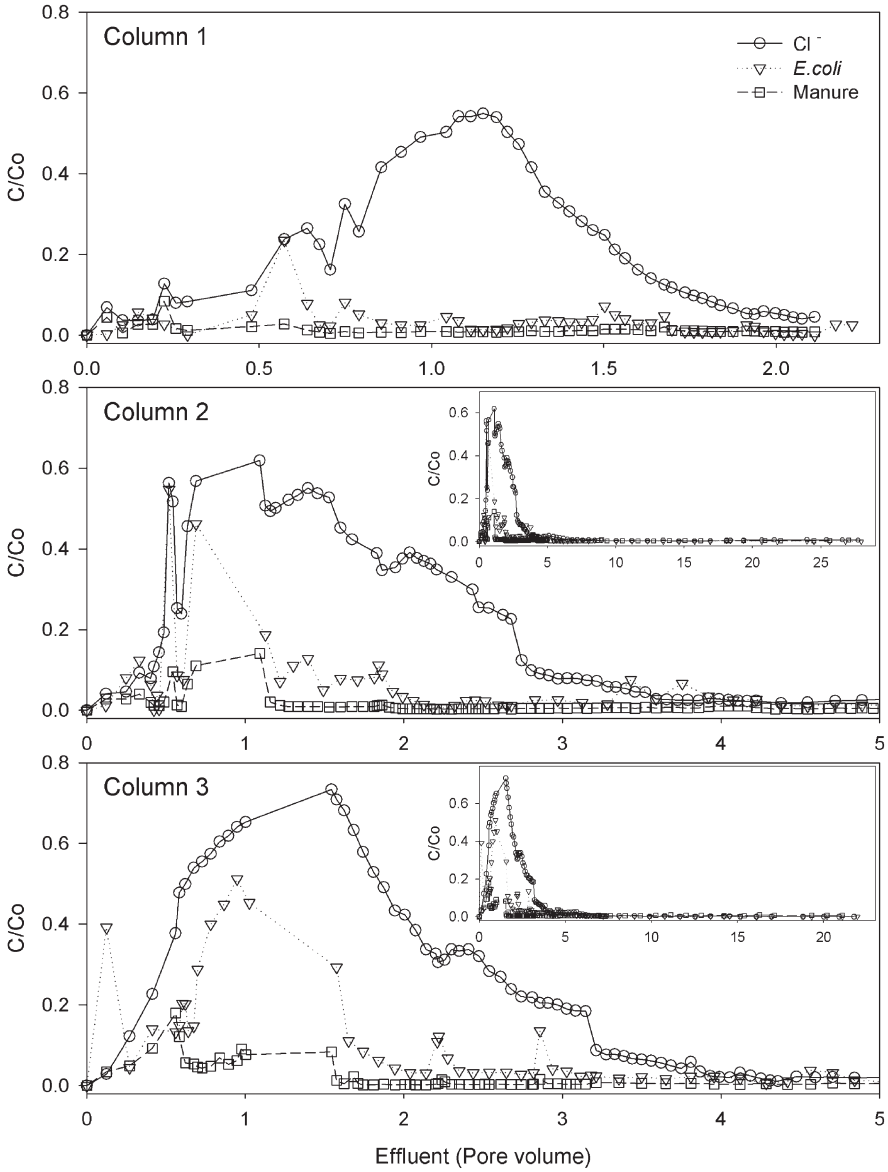

Fig. 2. Chloride, *Escherichia coli* and manure breakthrough curves. Flow velocities: 2.26 cm d^{-1} (column 1), 8.35 cm d^{-1} (column 2) and 9.28 cm d^{-1} (column 3).

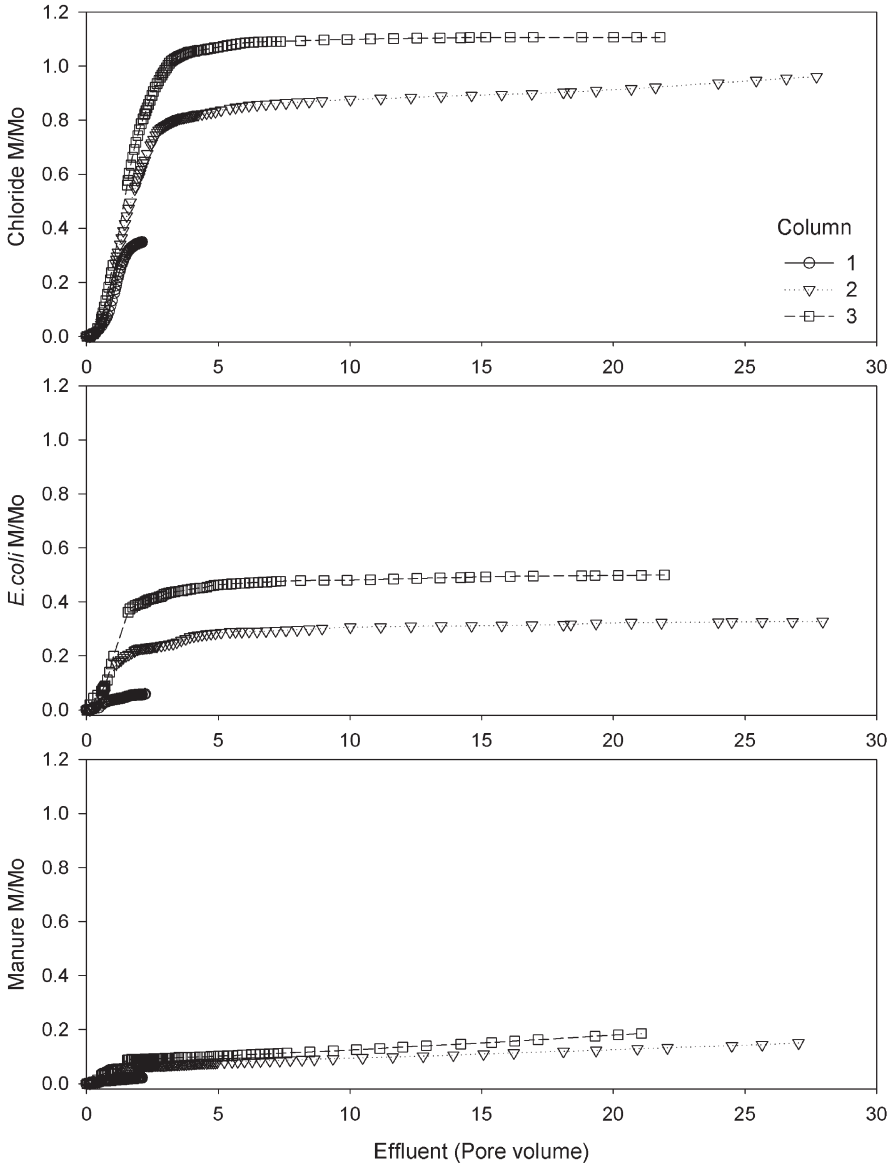

Fig. 3. Relative cumulative mass of chloride, *Escherichia coli* and manure in effluent.

soil columns after the total effluent volume was equal to half of column pore volume, one pore volume, and two pore volumes (Fig. 4a, 4b, and 4c, respectively). Breakthrough masses increased exponentially with an increase in flow velocities. Figure 4 shows that the same amount of effluent contained larger amounts of Cl^-, *E. coli* and manure in fast columns 2 and 3 than in the slow column 1.

The cumulative breakthrough of *E. coli* was more similar to that of manure particulates than to Cl^- in the slow column; paired values of leached relative mass for *E. coli* vs. manure were 0.011 vs. 0.009, 0.037 vs. 0.013, and 0.056 vs. 0.021, after 0.5, 1, and 2 pore volumes leached. On the contrary, *E. coli* leaching was faster than manure leaching, and was more similar to the chloride leaching in the fast columns (Fig. 4a). For example, 0.028 of *E. coli* and 0.027 of Cl^- mass passed the column 2 with 0.5 pore volume of effluent as compared with 0.047 of the manure relative mass. Later the transport of *E. coli* was somewhat retarded compared to that of Cl^- (Fig. 4b, 4c). The difference between *E. coli* and Cl^- mass in effluent increased and the difference between *E. coli* and manure mass decreased as more solution passed the columns.

Vertical distributions of volumetric water contents in columns after the experiment are shown in Fig. 5a. Water content was 1.5 to 2 times higher in top 2-cm layer than average values below this layer where no dependence on depth was observed. The smallest variability in water content (0.451 ± 0.026 cm^3 cm^{-3}) was obtained below the depth of 2 cm in column 2. Water content variability in columns 1 and 3 was identical, but average value in the column 1 (0.372 ± 0.063 cm^3 cm^{-3}) was less than in the column 3 (0.425 ± 0.076 cm^3 cm^{-3}).

Vertical distributions of soil bulk density in columns after the experiment are shown in Fig. 5b. The values of bulk density changed randomly (1.60 ± 0.16 g cm^{-3}) with depth in the upper 12-cm layer of soil column 1,

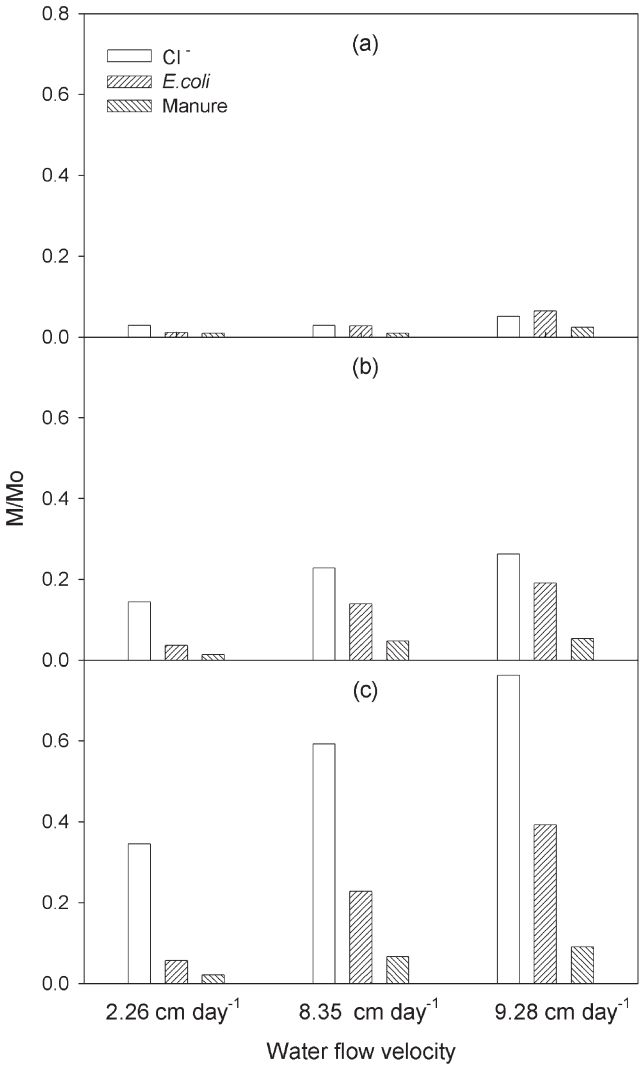

Fig. 4. Effect of water flow velocity on leaching of chloride, *Escherichia coli* and manure after 0.5 (a), 1.0 (b), and 2.0 (c) pore volumes passed through columns.

and decreased from 1.66 to 0.92 g cm^{-3} below the depth of 12 cm. The opposite trend in bulk density distribution was observed in columns 2 and 3. Bulk density decreased in columns 2 and 3 from the surface (0.98 and 0.90 g cm^{-3}) to the depth of 8 cm (1.67 and 1.66 g cm^{-3}). The average value of bulk density was less and variability in bulk density was higher in column 3 (1.37 ± 0.15 g cm^{-3}) than in column 2 (1.52 ± 0.10 g cm^{-3}) below 8-cm depth.

Profile distributions of viable *E. coli* are shown in Fig. 6. Maximum total content of *E. coli* was observed at the top of the slow column 1 and in the middle of the profile in fast columns 2 and 3. Profile distributions of *E. coli* in pore solution resembled the water content distributions below 0- to 2-cm layer in all columns. The determination coefficient of the linear regression of average water contents vs. the average bacteria contents in the liquid phase was $R^2 = 0.48$. The total bacteria contents in soils were similar to the contents associated with the solid phase. Table 2 shows *E. coli* fractions left in soil and found in the effluent. More bacteria were found in soil of the slow column 1. The ratio of bacteria content in pore solution to the attached bacteria amount decreased as the velocity increased.

Relationships between *E. coli* content associated with the liquid and solid phase after the experiment generally followed attachment-detachment isotherms found in batch experiments (Fig. 7). The *E. coli* attachment to the solid phase in the soil with high flow velocity (column 3) could be described satisfactorily with the Freundlich isotherm obtained for 40 000 mg L^{-1} manure solution in the batch experiment. For the soil with low flow velocity (column 1), the data from soil column layers were above the 20 000 mg L^{-1} batch isotherm (Fig. 7) corresponding to the attachment from solutions with manure concentrations lower than 20 000 mg L^{-1}.

DISCUSSION

Escherichia coli Attachment to Soil

Applicability of the Freundlich Eq. [1] to bacteria attachment to soil particle was earlier shown for fecal coliforms (Gantzer et al., 2001). These authors obtained values of $K_f = 55.3$ and $n = 1.07$ using the same experimental technique with a soil of the similar clay loam texture. Those values are reasonably close to the parameters of the Freundlich isotherm in this study in the absence of manure.

An increase in manure concentrations reduced bacteria attachment to soil (Fig. 1). The decrease in bacteria attachment could be caused by (i) modification of soil mineral surfaces by soluble manure organic and inorganic constituents; (ii) competition of dissolved organic matter and bacteria for adsorption sites; (iii) modification of bacteria surfaces by the dissolved organic matter. Some of those mechanisms have been observed in groundwater and sediment studies. Scholl and Harley (1992) observed an increase in bacteria adsorption after the removal of dissolved organic C from the bacterial suspension used in the adsorption experiment. The authors explained their results by competition between dissolved organic C and bacteria for positively charged surface sites on the sand. Johnson and Logan (1996) studied the effect of dissolved organic matter on Savannah River strain A1264 adsorption on quartz and iron (Fe)-quartz particles. They concluded that organic matter enhanced bacterial transport by increasing the negative surface charge of quartz and bacteria and made the bacteria-quartz interaction electrostatically unfavorable. It remains to be seen which mechanisms prevail in the effect of manure in solution on bacteria attachment to soils.

Column Transport

All BTCs had long tails in this study (Fig. 2 and 3). Tailing in BTC was observed in column studies of reactive solute transport and was attributed to (i) the adsorption kinetics and (ii) differences in mobility of water in different parts of pore space. Kinetics of straining was also suggested as a mechanism causing BTC

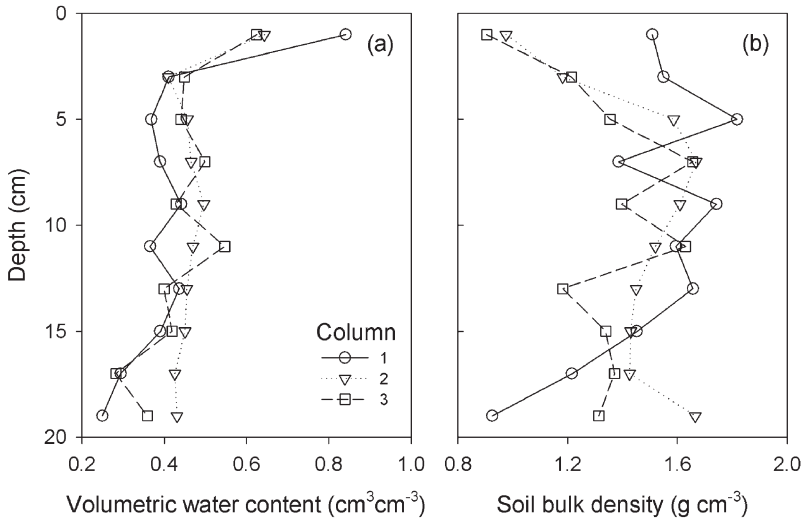

Fig. 5. Water content (a) and bulk density (b) distributions in soil columns after the experiment.

tailing in bacteria and colloid transport. The effect of all these mechanisms has been observed in column studies of bacteria transport in sand. Hendry et al. (1997) observed well-defined tails on BTCs of *K. oxytoca* percolated through water-saturated silica sand. The tailing was explained by both an irreversible and kinetically controlled reversible sorption. The estimated rate constant of the bacteria attachment was five times greater than the estimated detachment rate in their study. Later, Hendry et al. (1999) concluded that differences between the breakthrough concentrations of the bacteria and the conservative Cl^- tracer could be attributed to sorption. Fontes et al. (1991) created zones with different permeability in sandy columns and observed BTC tailing both for Cl^- and for bacteria due to the differences in mobility of water in different parts of the pore space. Wollum and Cassel (1978) observed considerable tailing in breakthrough curves of S*treptomycetes* spp. in sand columns. Anion BTCs did not have tails in the same study, and the authors hypothesized that microorganisms, unlike anions, might be trapped in small pores and a fraction of them subsequently released over time into water flowing through the porous medium.

The kinetics of bacteria and manure attachment-detachment should have a role in the BTC tailing in this work. Bacteria retention in soil columns was greater in column 1 with low flow velocity, than in columns 2 and 3 with high velocity (Fig. 7). That can be explained by substantially larger time that bacteria have had to attach to surfaces. Leached amounts of *E. coli* were greater than leached amounts of the conservative tracer Cl^- in columns 2 and 3 (Fig. 4a). This indicated that after half pore volume had been leached attachment-detachment equilibrium was not approached at high flow velocity. Much more time was available for the attachment to bacteria in column 1, and the relative mass of leached bacteria in this column was much smaller than that of chloride. Bacteria detachment also could be enhanced at high flow velocities. More *E.coli* was released from columns with high velocity after the pulse of manure and bacteria suspension passed through the soil. Attached bacteria can start to move with water flow when the external forces overcome the attractive ones. The differences in mobility of pore solution could also be a factor of tailing. When a part of pore water is relatively immobile compared to another part, a nonreactive tracer is transported mostly in mobile water, and slowly diffuses to and from immobile water. The BTCs are strongly skewed for such transport conditions. The Cl^- tracer in this work had a symmetrical BTC in the column with low flow velocity and skewed BTCs in columns with fast flow (Fig. 2). Therefore, substantial differences in mobility of different parts of soil water existed in columns with fast flow and could be partly responsible for the tailing on *E. coli* and manure BTC.

The cumulative breakthrough of *E. coli* and manure particulates was larger in fast columns compared with the slow column (Fig. 3). Such transport enhancement has been observed in experiments with pure culture bacteria transport in sand and disturbed soil columns (Wollum and Cassel, 1978; Smith et al., 1985; Tan et al., 1994; Banks et al., 2003) when columns were made as similar as possible, and the differences in velocities were imposed. The differences in flow velocities among the columns in this work reflected natural field soil variability. Soil porosity in column 1 with low velocity was smaller (0.420 $cm^3\ cm^{-3}$) than in columns 2 and 3 (0.433 $cm^3\ cm^{-3}$ and 0.445 $cm^3\ cm^{-3}$). Our hypothesis is that the differences in velocities are related to differences in soil structure, larger velocity corresponded to greater macroporosity. Soil was well structured in this study (Table 1) and the presence of macroporosity should be expected. The role of macroporosity on bacterial transport in soil has been repeatedly discussed. Bitton et al. (1974) observed bacterial concentration peaks of the BTC earlier than the influent replaced one pore volume in a sandy soil column. They concluded that the mobile phase, which transported the bacteria, was smaller than the water content of the column. Wollum and Cassel (1978) concluded that bacteria transport occur primarily through the interconnected large pores.

Unlike results reported in the previous work of Shel-

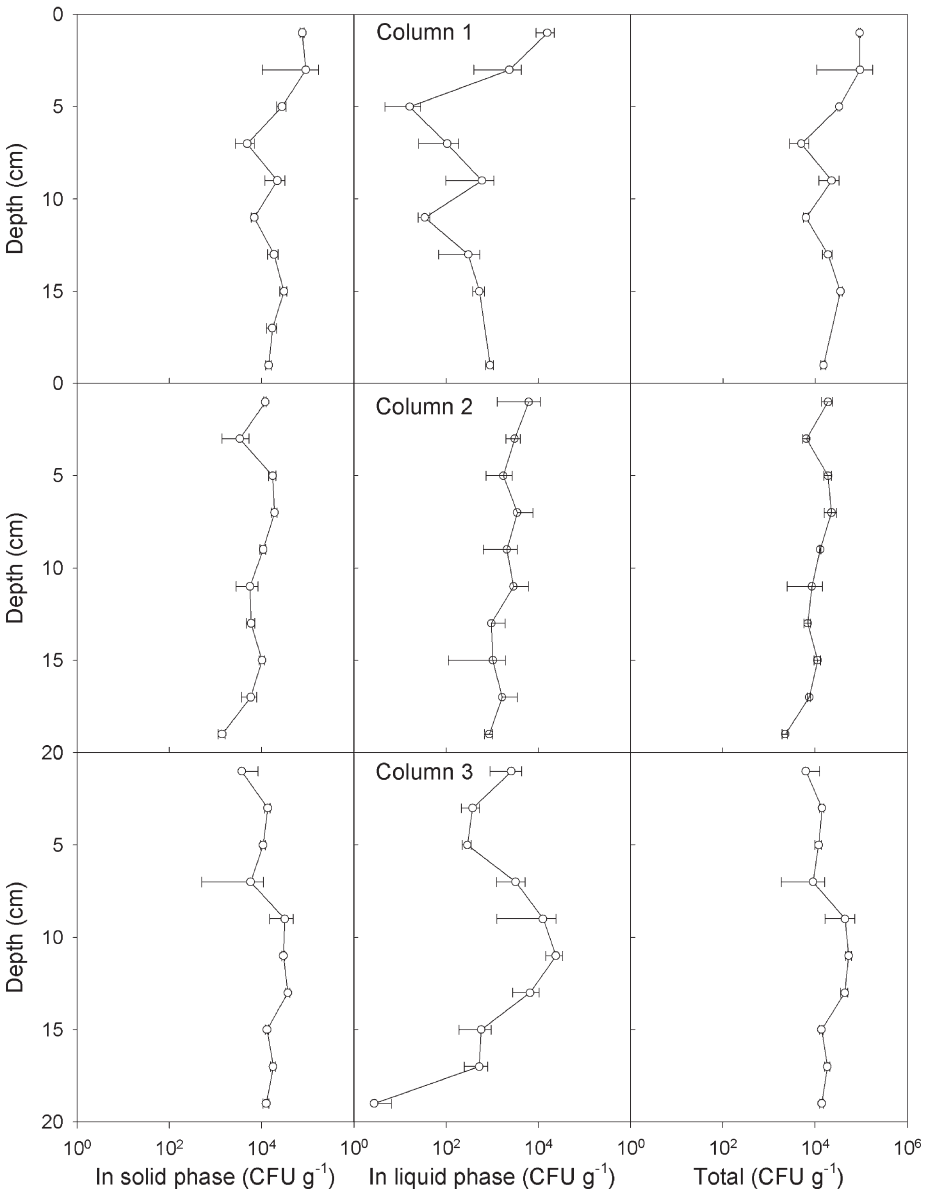

Fig. 6. ***Escherichia coli*** **distribution in columns after the experiment.**

ton et al. (2003), breakthrough of bacteria and manure was not similar (Fig. 3 and 4). At early stages of the breakthrough, a substantial amount of bacteria moved faster than manure in fast columns. At the late stages of breakthrough, relative concentrations of manure colloids stabilized at low levels that were sufficient to provide a continuous growth of cumulative leached manure up to 27 pore volumes. One reason for that could be a graduate dissolution of initially relatively large manure particulates that became strained in columns. Bacteria concentrations have also stabilized at low levels in late effluent portions. However, those levels were so low that they did not affect the cumulative breakthrough (Fig. 3). At least at late stages of the transport, manure particulates either did not serve as a carrier of bacteria, or transported bacteria in small amounts.

Profile distributions of viable *E. coli* in fast-flow columns did not show a significant trend of decrease with depth (Fig. 6). Large amounts of infiltrated water could not leach bacteria from the columns. Bacteria could probably enter parts of pore space that sheltered them and the low temperature in the experiment slowed down predation and prevented the die-off. Unlike the fast columns, the slow column had a peak of bacteria concentrations in the layer of loose soil at the top of the column. We hypothesized that manure particulates could settle in this layer and serve as an abode and a food source for *E. coli*. Manure accumulation on the surface of the slow column was visible, and data in Table 1 show a substantial difference in organic C content between column 1 and columns 2 and 3. *Escherichia coli* distributions below the loose top layer bore similarity with water content distributions. Higher water contents corresponded to higher bacteria contents, that is, in the middle part of the column 3; the lowest water contents in the bottom of the columns 1 and 3 corresponded to the

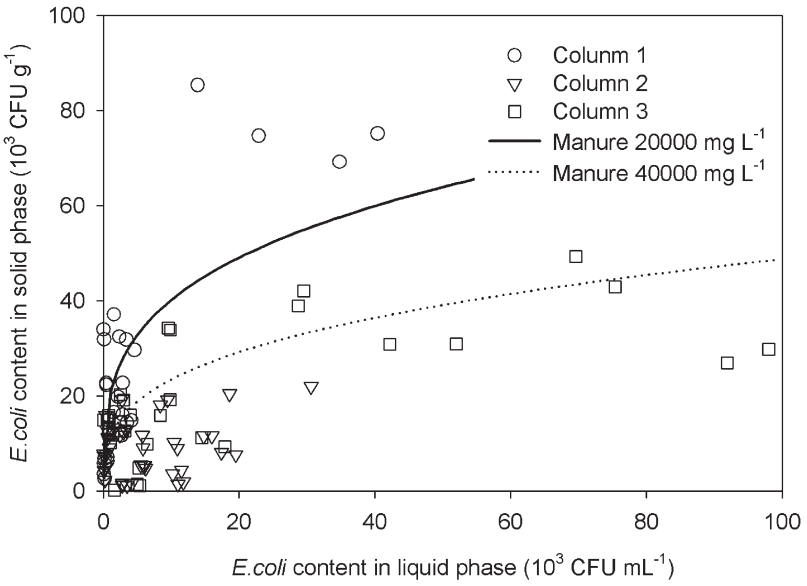

Fig. 7. ***Escherichia coli*** **distribution between liquid and solid phase; symbols: after transport experiments, lines: Freundlich isotherms from batch experiments.**

lowest bacteria concentrations in the profiles. In water-saturated columns 2 and 3, higher and lower water contents corresponded to looser and denser soil layers, respectively (Fig. 5). Looser soil layers might have a relatively large stagnant fraction of pore water where bacteria could be retarded.

Relationships between bacteria associated with the solid and liquid phase in Fig. 7 resembled the attachment isotherms in Fig. 1. Columns were disassembled during one day at the same temperature as the attachment isotherms were measured, and there was some time to approach attachment-detachment equilibria. The isotherms in Fig. 1 show that the lower the manure concentration the stronger the attachment. Based on the cumulative breakthrough, we assumed that manure content in the pore solution was less in column 1 (Fig. 3), and that caused the increase in bacteria attachment to soil particles compared with the other two columns. The data on equilibriums in columns after experiments indicate that there were no substantial differences in attachment among the columns. This underscores the importance of the velocity rather than attachment on the observed differences in column transport.

This study demonstrates that spatial variability in soil structure, resulting in large variations of pore water velocity, can cause substantial differences in transport of manure particulates and bacteria. Macroporosity appears to be responsible for the fast breakthrough of a relatively large fraction of applied bacteria and for tailing in breakthrough curves. The soil matrix serves as a straining and adsorbing media having relatively larger effect on the transport when the water flow is slow.

CONCLUSIONS

Chloride, *E. coli* and manure particulates were transported differently by water flow in the soil columns, and the transport was strongly affected by water flow velocity. Dissolved manure may decrease the *E. coli* attachment to soil particles. This study indicates that enhanced bacterial transport may happen in the soil with well-developed macropore system as a result of intensive rainfall in a short time period after manure application.

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